RESEARCH REPORT

1. Proposal number: 2009B1918

2. Title of experiment: Thin filament myopathies: Understanding the molecular mechanisms using X-ray diffraction

3. Project leader: Julien Ochala, Ph.D., Department of Clinical Neurophysiology, Uppsala University (Sweden)

4. Beamline used: BL45XU (small-angle scattering station)

5. Research background and purpose: Since their first discovery in 1995, more than 140 different mutations in genes coding thin filament proteins have been identified. They are notably located in TPM2 and NEB genes encoding β-tropomyosin and nebulin, respectively. These mutations are associated with severe muscle weakness and a range of other clinical and histopathological features known as thin filament myopathies.

The specific aim of the present proposal was to define the molecular mechanisms underlying muscle weakness in thin filament myopathies by investigating how two different mutations specifically alter thin filament behavior.

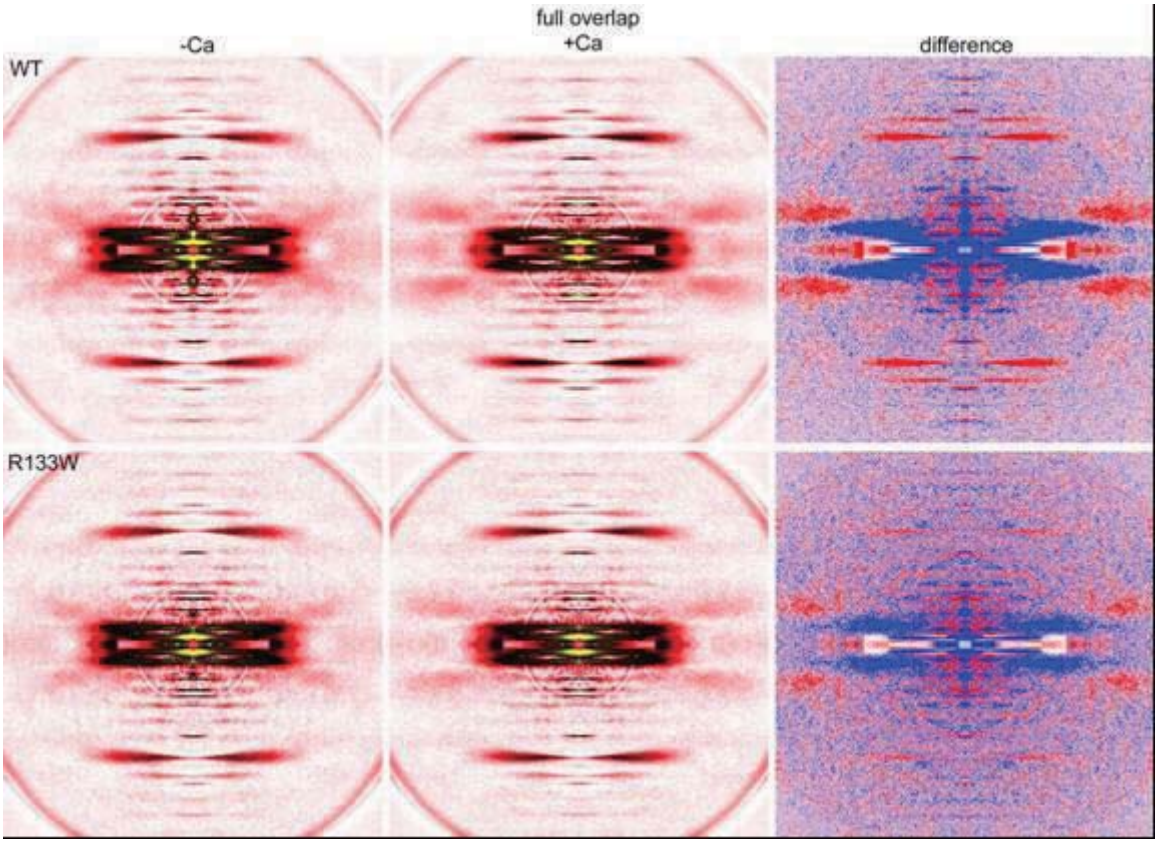
6. Experimental methodology: We performed X-ray diffraction experiments on membrane-permeabilized muscle fibres coming from biopsy sample specimens (i) from patients with a thin filament myopathy and carrying a β-tropomyosin mutation (R133W) or a nebulin mutation, and (ii) from healthy controls. The experiments were carried out using the SPring-8 synchrotron radiation facility (Harima, Hyogo, Japan). On the day of experiment, muscle fibres were dissected and mounted in arrays of 30 fibres. X-ray diffraction patterns were recorded for each array of 30 membrane permeabilized fibres in relaxing (low [Ca^{2+}]) and activating (high [Ca^{2+}]) solutions at full-overlap (2.70 μm) and non-overlap (> 3.60 μm) by using a cooled CCD (charge-coupled device) camera (C4880, Hamamatsu Photonics, 1000 × 1018 pixels) in combination with an X-ray image intensifier (V5445P, Hamamatsu Photonics). The wavelength was 0.09 nm, and the specimen-to-detector distance was ~2 m. To compensate for the relatively small dynamic range of the detector, absorber masks made of aluminum and copper were placed at the center of the image intensifier. The exposure time was ~2 s, and usually several to tens of patterns were summed to obtain a final image to be analyzed. The four quadrants of the image were folded after correction for the fiber inclination, and the background was subtracted.

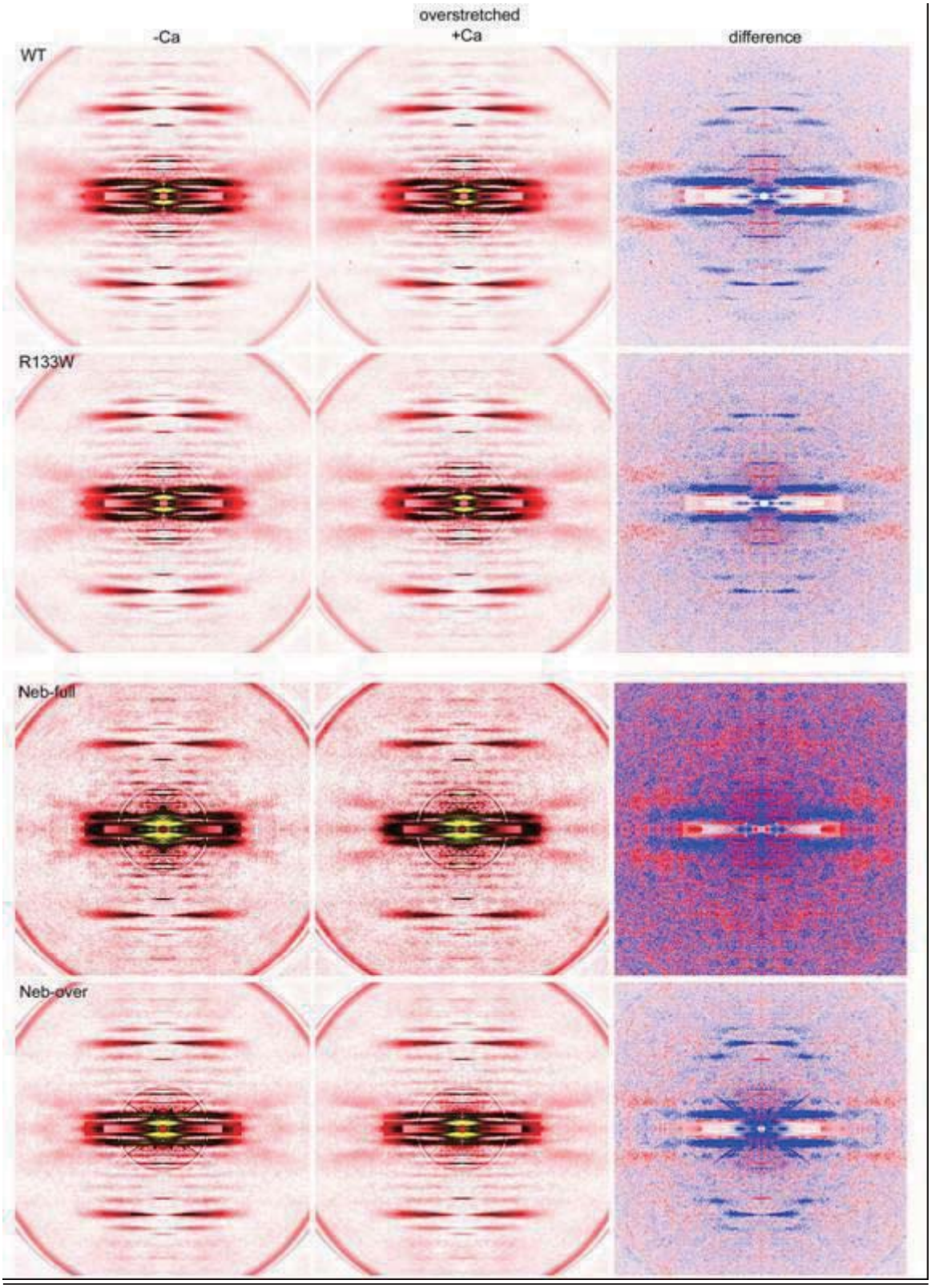
7. Research results: In control fibres at full-overlap, the 6th ALL is enhanced by 11% upon addition of Calcium whereas the 2nd ALL is enhanced by 20%. The 7th is also enhanced. In control non-overlap fibres, the profile of the 6th ALL slightly shifts outward, and its intensity is almost unchanged after addition of Calcium. The 2nd ALL is enhanced

by 15.5% upon addition of Calcium. This is consistent with previous data and supports the idea that the thin filaments need attached cross-bridges, as well as Calcium, to be fully activated.

The fibres carrying the β-tropomyosin mutation (R133W) at full-overlap shows weaker enhancement of the 2nd ALL (13.5%) as compared with controls. This means that the thin filaments are not fully activated even at saturating Calcium. The 6th ALL is enhanced by only 2.5%, demonstrating that the inhibition of tropomyosin movement causes an inhibition of actin conformational change. The enhancement of the 7th ALL is not evident. In non-overlap fibres (R133W), the enhancement of the 2nd ALL is also weaker (12.7% vs. 15.5% for controls) suggesting that thin filaments activation is due to calcium disrupted signal and cross-bridges non-attachment.

In fibres carrying the nebulin mutation (NEB) at full-overlap, the enhancement of the 2nd ALL seems weaker than in control (16% vs. 20%). This means that the thin filaments are not fully activated even at saturating Calcium exactly as for R133W. However, in non-overlap fibres (NEB), the enhancement of the 2nd ALL is indistinguishable from controls, suggesting that thin filaments activation is only due to cross-bridges non-attachment.




overstretched
-Ca
+Ca
difference
WT
R133W
Neb-full
Neb-over

8. Current/future issues: All the results taken together are promising and represent important features for the understanding of the thin filament myopathies.

9. Status of publication: Data will be soon sent to a scientific journal.

10. Key words: Muscle disease, gene mutation, tropomyosin, nebulin, structure and function.